

INHERITED VARIATION ARISING DURING VEGETATIVE REPRODUCTION IN *PARAMECIUM AURELIA*¹

DANIEL RAFFEL²

(From the Zoölogical Laboratory of the Johns Hopkins University and the Marine Biological Laboratory, Woods Hole, Mass.)

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INTRODUCTION

Jennings (1908), and Jennings and Hargitt (1910) have shown that populations of *Paramecium* which are produced from a single individual are uniform in size, form and fission rate. Other investigators have since confirmed these findings in other genera of Protozoa. Increased variation and the production of different stocks have been found to be produced by conjugation by Jennings (1913), Raffel (1930), Jennings, Raffel, Lynch, and Sonneborn (1932). Other investigators, Middleton (1918), Mast (1917), and Jollos (1921), have found inherited variation arising during vegetative reproduction which they attributed to the action of environmental agents. However, most investigators agree that when a clone of *Paramecium* is cultivated under uniform conditions it is usually decidedly uniform. An excellent review of the literature has recently been given by Jennings (1929).

¹The present contribution is one of a series of studies on the genetics of conjugation and reproduction in Protozoa, in progress by H. S. Jennings, his associates, and students. The author wishes to record his indebtedness to Professor Jennings for his interest and advice throughout the course of the work.

²Fellow of the National Research Council.

This paper presents the results of a study of a clone which sporadically produced branches, or lines, which differed markedly from the original type. These lines, which will be described in detail later, differed in many respects from the unaltered lines and these differences persisted for long periods under identical conditions. None of the altered lines was ever observed to revert to the original type although some were cultivated for nearly 100 generations.

MATERIALS AND METHODS

The organisms used in this investigation were members of a clone of *Paramecium aurelia* descended by fission from a single ex-conjugant (known as 128a) obtained July 15, 1930, in the course of another investigation.

The methods of cultivation employed were the same as those used in the author's (Raffel, 1930) earlier work except that it was found necessary to add a small quantity of the bacteria to the fresh culture fluid each day. The culture medium consisted of a fluid containing KNO_3 , 0.5 gram; K_2HPO_4 , 0.06 gram; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.02 gram; FeCl_3 , 0.001 gram; H_2O , 1 liter. To this solution cultures of an alga, *Stichococcus bacillaris*, and a bacterium, usually *Achromobacter candidans*, were added. The paramecia were cultivated in this suspension under bacteriological conditions.

DESCRIPTION OF THE ALTERED AND UNALTERED LINES OF THE CLONE

1. *Origin.*

The clone 128a differed markedly from the other clones isolated from a group of individuals undergoing conjugation July 15, 1930. It was found to have a characteristic form and size quite diverse from the form and size of the other clones which were studied. It also differed from most of the other clones in its slow rate of reproduction. For these reasons it was selected as one of the few clones to which was devoted further intensive study. Later study has shown that it also differed from most of the other clones in its tendency to give off branches quite different from the main clone and in its ability to withstand adverse environmental conditions.

On August 4, 1930, twenty-two days after this clone was isolated, two of the twenty-four lines which were being cultivated became radically altered in ways to be described below. Three other lines became altered in a similar manner during the next three days. These five lines were discarded on August 15. During the five days following August 29, seven more lines became altered in a similar manner. These were

cultivated until September 18, when they were discarded. No other altered lines appeared until early in November, but from that time until June of the following year, they were produced very frequently.

2. Comparison of the unaltered and altered lines.

The typical individuals of this clone, hereafter referred to as the unaltered lines, differed from the altered lines in the following ways:

(a) Size. One of the most striking differences between the two groups was the difference in size. This difference is well illustrated by Fig. 1, which shows typical specimens of each group under identical

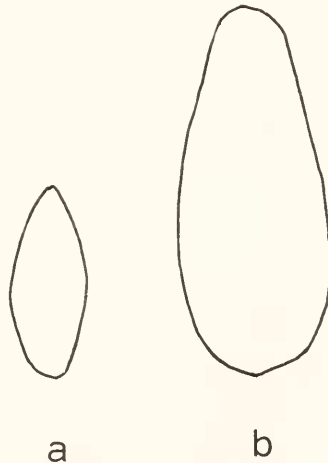


FIG. 1. Camera lucida drawings of typical individuals of (a) unaltered and (b) altered lines.

conditions. As can be seen from this figure the individuals of the altered lines were more than twice as long as those of the unaltered lines. This marked size difference was a matter of continuous daily observation.

(b) Form. The two branches of clone 128a differed markedly in form. The unaltered individuals of this clone were thick in the central region and tapered to points at each end. They were quite pale and usually had a dark food vacuole in the anterior tip. The altered individuals were, on the other hand, of the typical *P. aurelia* form, *i.e.*, somewhat pointed anteriorly, rounded posteriorly with their greatest breadth about two-thirds from the anterior end. Usually they had many food vacuoles distributed chiefly in the posterior part of the organism. These differences in form as well as the size differences are shown clearly in Fig. 1. This figure shows typical adult individuals of

both kinds living under identical conditions and drawn at the same time. The differences in form and also the differences in size persisted even during the periods of depression when the fission rates of the two groups approached one another. At these times the two groups could be readily distinguished by their appearance.

(c) Fission Rate. The altered lines differed from the unaltered lines greatly in their rates of reproduction. The clone originally was a very slowly dividing one with a mean fission rate much below one divi-

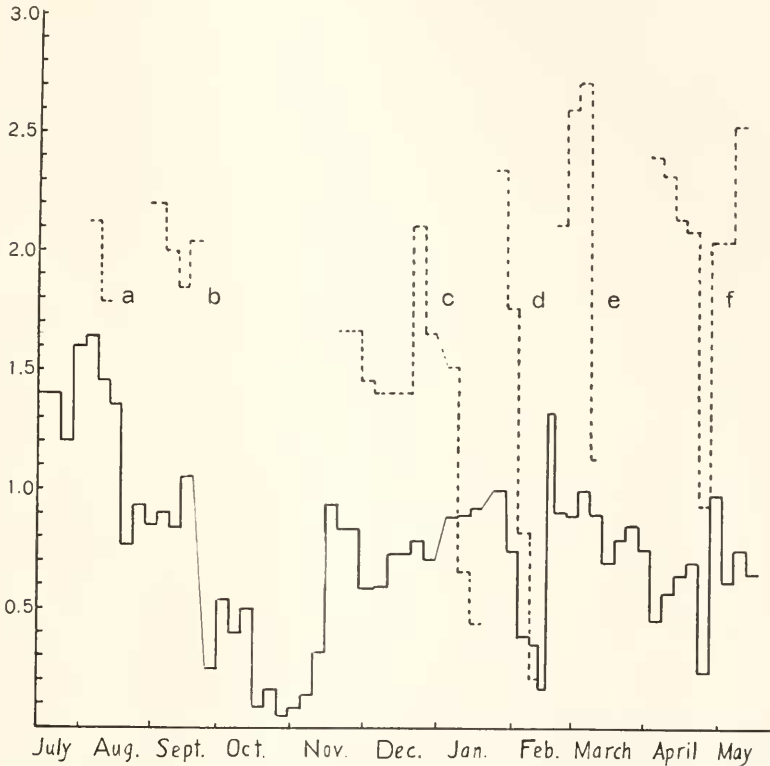


FIG. 2. Average daily fissions of unaltered (solid line) and of altered (dotted lines) lines plotted in periods of approximately five days each.

sion per day. The altered lines, on the other hand, divided very vigorously and some of them averaged well over two fissions per day for periods exceeding two weeks. This difference in fission rates is illustrated in Fig. 2. The solid line in Fig. 2 shows the mean daily fission rates for the original clone from July 15, 1930 to May 17, 1931, plotted in approximately five-day periods. The dotted lines represent the average daily fission rates of six different groups of altered lines which

were studied. These are also plotted for the most part in five-day periods. From this figure it is evident that the altered lines differ markedly from the unaltered lines in their rates of reproduction.

(d) Resistance. The altered lines of clone 128a were much less resistant to unfavorable conditions than were the unaltered lines. This can be readily observed from Fig. 2 which shows that in nearly every period of depression the altered lines suffered a decrease in fission rate earlier than the unaltered lines, and that the extent of the depression was greater in the altered lines. For example, group (c) Fig. 2 declined in fission rate from 1.26 to 0.43 fissions per day between January 10 and January 20, while the unaltered lines showed an actual increase from 0.88 to 0.93 fissions per day during the same period. Similar though not so great differences can be found by comparing groups (d), (e) and (f) with the unaltered lines which were cultivated simultaneously with them.

We have seen then that the clone 128a produced branches during vegetative reproduction which differ markedly in size, form, fission rate, and resistance from the unaltered lines. The differences in size and form persist even during periods of depression during which the fission rates of the two groups were similar. These differences are as great as any found by Jennings, Raffel, Lynch, and Sonneborn (1932) in their extensive study of diversities between biotypes produced by conjugation. The altered lines persisted for long periods and none has ever been observed to revert to the original condition although many thousands of individuals have been examined.

EXPERIMENTS

1. *Regularity in the production of altered lines.*

The remaining parts of this paper deal with experiments designed to discover whether any regularity was apparent in the production of these altered lines, and whether after conjugation these unlike branches of the same clone would produce similar or dissimilar populations. In order to learn more about the occurrence of the altered individuals and to ascertain whether there is any regularity in their production, the following experiments were performed:

From November 3rd to 19th the progeny of one individual of the clone was expanded to form 336 lines of descent whose relationships were known. During this period some of the lines became altered. These, because of their faster rate of reproduction, gave rise to the greater part of this population. After the desired number had been obtained, the organisms were cultivated under identical conditions for

ten days. During this period the lines which were altered at the beginning passed through from 1–21 generations (all but one of the 226 such lines which lived until November 29 passed through from 11–21 generations), while the unaltered lines passed through from 4–9 generations. As is shown in Fig. 2 (c), the mean daily fission rate for the altered lines is 1.67 as compared with 0.83 fissions per day for the unaltered lines.

On November 29 all except one of each of the altered and unaltered lines were discarded. Then each of these was expanded to a population of 168 individuals. These two populations were then cultivated for ten days from December 11–21, during which time they showed great diversity in their size, forms and fission rates (*see* Table I). Although

TABLE I

Distribution of total fissions December 12–21, 1930, of populations derived from (a) a single individual which manifested the altered set of characteristics on November 18, and (b) from an individual of the original type.

Fissions	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	Mean
Altered lines	1			1		1	3	3	7	7	2	3	9	12	12	24	13	10	4	13.1
Unaltered lines	0	1	5	10	12	17	18	8	6	5	5	5	6	5	4	3	2			7.2

the altered lines were much depressed at this time because of unfavorable environmental conditions or endomixis, the population derived from the altered individual produced no individuals which were like the unaltered lines.

Figure 3 is a genealogical chart showing the relationships of the lines descended from the unaltered individual, with their numbers of fissions during the ten days following December 11. These lines were all of the original type on December 11; but, as is shown, many of them, represented by the filled-in circles on Fig. 3, became altered subsequently.

From the data given in Fig. 3, an attempt was made to ascertain whether or not there is any regularity or rule in the production of altered stocks. That is, if a given line is altered, are the lines which are separated from it by fewest fissions more likely to be altered than lines separated by more fissions or not; or is any other rule to be found which would describe the production of the altered lines? To investigate this question the coefficients of correlation were obtained for the total number of fissions between December 12 and December 21, of lines represented on Fig. 3 which were separated on December 11 by one, two and three fissions. The lines which were separated by but one fission at this time showed a correlation of 0.56 ± 0.06 ; those separated by two fissions a correlation of 0.23 ± 0.12 ; while those separated by three fis-

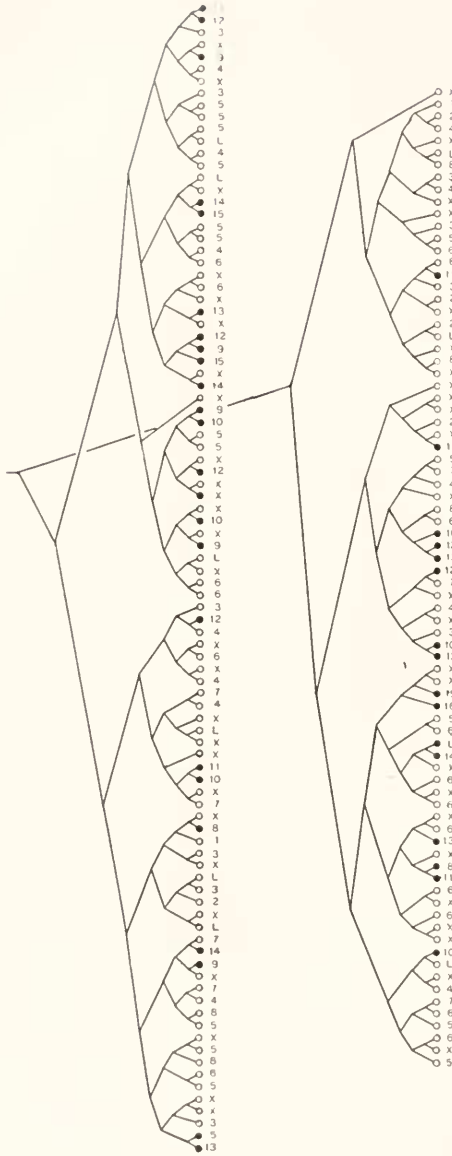


Fig. 3. Genealogical chart showing the relationships of the lines derived from an unaltered individual between November 29 and December 11, 1930, with the number of fissions of each line from December 12-21 inclusive. The lines were all unaltered on December 11. Those which remained unaltered are represented by open circles, those which became altered by solid circles.

X indicates that the line died before December 21.

L indicates that the line was lost.

sions gave a coefficient of correlation of 0.48 ± 0.06 . Thus there is apparently no relation between the number of fissions by which lines are separated and the fate of the lines. The decrease in the coefficients of correlation of the lines separated by two fissions as compared to those which are separated by only one appears to be meaningless when we find that those separated by three fissions do not differ significantly from those separated by only one fission. A careful study of the records, however, shows that when two lines are derived from a common ancestor, one or two generations before one of them becomes altered the other usually becomes altered in a similar manner at nearly the same time. This occurred frequently throughout the entire six months that this subject has been intensively investigated.

The experiments and observations which were made with the object of discovering any regularity or rule in the production of altered lines did not give any evidence of any such regularity. They did show, however, that if a line becomes altered, lines which were derived from the same individual not more than two generations previously generally became altered at the same time or at nearly the same time.

2. Effect of conjugation in the diverse lines.

In another paper Jennings, Raffel, Lynch, and Sonneborn have shown that when two diverse clones conjugated they produced two very diverse populations. In the two clones intensively studied, although each population showed great variation in fission rates, the mean fission rate of each was not very different from the fission rate of the clone which produced it. In the particular cases which they studied the mean fission rates of the two populations differed in the same direction and to approximately the same extent from the mean fission rates of the clones from which they came. In the present investigation we have two races which differ more than the two clones studied by Jennings, Raffel, Lynch, and Sonneborn. These two races are, however, not different clones but are branches of the same clone; that is, they are descended by vegetative reproduction from a common ancestor. Will these two very diverse races also produce by conjugation populations which are on the whole diverse, or will they, unlike diverse clones, produce populations which are similar? In order to answer this question typical lines of the two races of this clone were chosen and expanded in isolation drop cultures until large numbers of both types were available. Then conjugation was induced in both branches of the clone and 105 pairs of conjugants and 96 non-conjugants were taken in each group. In the case of the slowly reproducing unaltered lines care was taken to choose among the non-conjugants some split pairs and others which were be-

ginning to conjugate. These four sets of organisms were then cultivated side by side for ten days after the last ex-conjugants were isolated.

The results of this experiment are shown in Figs. 4 and 5. Figure 4 shows the course of the mean daily fission rate of the two groups of non-conjugants; and Fig. 5 shows the same for the ex-conjugants. As can be seen, the non-conjugants of the unaltered lines continued to reproduce at the rate of approximately 0.9 fissions per day, while the ex-conjugants from this group reproduced much more rapidly, reaching at the end of the experiment a rate of 1.6 fissions per day. This progressive increase in fission rate was due entirely to the dying of the many abnormal lines which were produced by conjugation. Ninety-three non-viable lines were produced. Only 10 of 210, or 4.8 per cent, of the ex-conjugant lines resembled the parent race in fission rate.

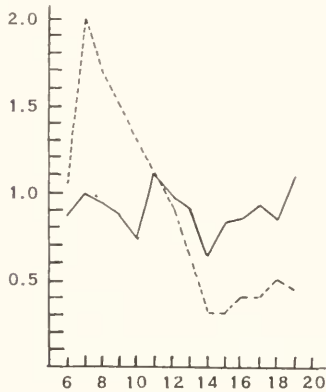


FIG. 4

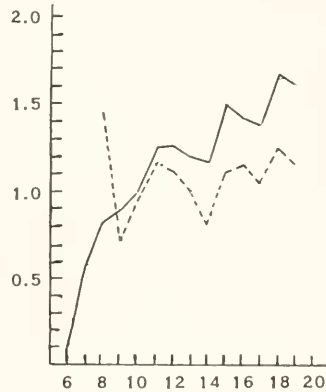


FIG. 5

FIG. 4. Average fissions on successive days of non-conjugant lines of the unaltered (solid line) and altered (dotted line) groups.

FIG. 5. Average fissions on successive days of the ex-conjugant lines of the unaltered (solid line) and the altered (dotted line) groups.

Among the 96 lines of the non-conjugants, 29 became altered and 24 lines died out during this period.

The results obtained from the altered lines were quite different. The non-conjugants at the beginning were very healthy and vigorous, reproducing at the rate of two fissions per day the second day after conjugation and nearly as rapidly the next day. From then on they declined rapidly in vigor—the fission rate falling to between 0.3 and 0.5 fission per day, accompanied by a great increase in mortality. The ex-conjugants, which were also very vigorous at first, with a fission rate of 1.4 for the first day, declined to approximately one fission per day

in spite of the death of the 90 non-viable lines which had been produced by conjugation.

Because of the depressed conditions of the organisms during this experiment it seemed advisable to repeat it under more favorable conditions. Great difficulty was experienced in trying to obtain the conjugants from the unaltered lines as these were constantly producing organisms of the altered type, which in a short time would greatly outnumber the type which the culture originally contained. Finally, in order to know which type of organisms was conjugating in the cultures of the original group, split pairs were taken at the same time as the conjugants. Thirty-three per cent of all of the organisms obtained from split pairs were of the original type. However, no pair among the split pairs was composed of two organisms of this type. It is very probable that this was due in a great measure to the subsequent altera-

TABLE II

Distribution of fissions during the first five days after conjugation of ex-conjugants derived from (a) altered lines and (b) unaltered lines of clone 128*a*.

Fissions	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	No. of lines	Mean
Altered lines	4	0	1	1	2	3	3	9	10	14	17	10	24	33	19	1	151	10.5
Unaltered lines	0	4	2	3	4	11	6	6	8	8	19	18	38	46	24	19	216	10.9

tion of one member of the pairs, as altered lines were being produced rapidly at this time.

The results of the conjugations in the two branches were as follows: The unaltered lines gave rise to a population very different from themselves. The mean fission rate of 2.2 fissions per day is much greater than the 0.62 fission per day of the non-conjugants which lived through the same period. The ex-conjugants were somewhat less viable than the non-conjugants of this group; 22.3 per cent of the former died during this period as compared to 18.9 per cent of the non-conjugants. Only 11 of the 278 ex-conjugant lines or 4 per cent had the shape and size of the unaltered lines.

On the other hand, the altered lines gave a quite different result. The ex-conjugant lines were much less viable than the non-conjugants; 34.7 per cent of the lines derived from ex-conjugants died, while there was no mortality in the non-conjugants. The mean fission rate of the ex-conjugants was 2.1 fissions per day as compared with 2.3 for the non-conjugants. No single individual among the ex-conjugant lines obtained from the altered group had the size or form of the unaltered lines. A comparison of the two groups of ex-conjugants with respect to their fission rates is given in Table II. This table shows the great

similarity between the fission rates of the two populations which have the same mode and means which differ very slightly.

These results differ markedly from those obtained by Jennings, Raffel, Lynch, and Sonneborn, on the effects of conjugation in their two diverse clones. The two populations produced after conjugation by the two diverse branches of the same clone, in the investigation presented here, are nearly identical with respect to their fission rates. The mean fission rates of both populations are nearly equal to the mean fission rate of the altered population. In the case of the altered lines this result is in accordance with the results obtained by earlier workers. Jennings (1913) and Raffel (1930) found that conjugation within a clone results in a population showing much variation, with a mean fission rate slightly lower than that of the parent clone, and an increase in mortality. In the case of the unaltered lines of this clone the effect of conjugation is to produce a population quite different from the non-conjugant population. The mean fission rate is increased to more than three times the mean fission rate of the non-conjugants and the size and shape of 96 per cent of the ex-conjugants is altered. Thus the unaltered lines of clone 128a are affected by conjugation in a way quite unlike most of the clones which have previously been described.

It is obvious that the two very diverse branches of clone 128a are affected quite differently by conjugation. Instead of giving rise to two diverse populations as did the two diverse clones investigated by Jennings, Raffel, Lynch, and Sonneborn, they produced, after conjugation, populations nearly identical in fission rates and appearance. This indicates certainly that the inherited diversity which appeared during vegetative reproduction in this clone differs in some way from the diversities between the clones studied by Jennings, Raffel, Lynch, and Sonneborn.

DISCUSSION AND CONCLUSIONS

The inherited variations which arose in this clone during vegetative reproduction differ markedly from those which are produced by conjugation. The altered lines which were produced were similar to one another in all respects and they were quite different from the original type, while conjugation produces from a single clone a large number of clones which differ much or slightly from one another so that a more or less continuous series is produced. The differences between the two branches of this clone are to a great degree eliminated by conjugation while diverse clones produced by conjugation usually yield after subsequent conjugations populations which are similar to themselves in their fission rates and other characteristics. This suggests that the basis of

heritable variation found in this clone is different from that of the variation produced by conjugation.

The increase of variation after conjugation and the cytological details of that process have led to the conclusion that conjugation involves biparental inheritance and that the increased variation produced by conjugation is brought about by a recombination of genetic factors. The inherited variation reported in this paper, however, does not seem to arise from such recombinations. It differs from the variations produced in that manner as set forth above; and in order to ascribe its origin to such recombinations it would be necessary to postulate some such process for this particular clone only. Therefore, it seems that recombinations must be dismissed as a possible explanation of the origin of this variation.

That the genetic constitution of the original clone must contain a number of heterozygous pairs of genes is evident from the results of conjugation between members of the unaltered branch. In order that a particular clone should produce, when inbred, a population only 4 per cent of which resembles the parent, it is necessary that the clone should be heterozygous for four or five genes. Therefore, we can assume that a heterozygous condition of four or five pairs of genes is necessary for the production of the unaltered type of this clone. A mutation then of any of eight or ten genes would produce an altered line in this clone. It is possible, however, that the observed altered lines could only be produced by the mutation of any of the four or five "mutant" genes to the "normal" condition (as a mutation of the "normal" gene might be lethal when homozygous). The evidence leads to the conclusion that the production of altered lines in this clone is probably caused by the mutation of one of the members of the four or five pairs of heterozygous genes.

This conclusion explains all of the phenomena observed. There is no regularity in the production of the altered line—no regularity would be expected of gene mutations. The altered lines never produce the original type after conjugation—as it is not heterozygous for all five pairs, it cannot produce progeny which are heterozygous for all five pairs.

The only question which this conclusion raises is the frequency of gene mutation in *Paramecium*. This is to be the subject of a subsequent paper, but it might be said here that this explanation of the questions raised by this investigation throws some light on other questions. Jennings, Raffel, Lynch, and Sonneborn found that clones differ in their uniformity. Others have found variation between clones in mortality, etc. If gene mutations are comparatively frequent in *Paramecium*, it

can account for the cases of inherited variation reported by other workers as well as the unexplained mortality that occurs in all isolation culture work. It, furthermore, can explain the differences in mortality which are observed in different clones. The only alternative to this explanation is that the original clone contained a detachable translocation which was lost in the production of the altered branches. The former explanation seems preferable because it explains the numerical relationships found in this investigation as well as the other phenomena which have been mentioned; and also because much evidence has subsequently been obtained which indicates frequent mutations in *Paramecium*. A more detailed treatment of these questions will be presented in the near future.

SUMMARY

The clone of *Paramecium aurelia* studied in this investigation produces branches which differ from the original clone in many respects. They are larger, have a different form, reproduce at a greater rate, and are less resistant to unfavorable conditions than the original type. These branches consistently manifest their diverse characteristics and none has ever been known to revert to the original type in any of the cases which were studied. No rule or regularity was found in the production of the altered lines except that when a line became altered, lines which were separated from it by only one or two fissions usually became altered at the same time or nearly the same time. Unlike diverse clones, these diverse races of the same clone give after conjugation populations which are on the whole very similar. The unaltered type gave after conjugation only a very small proportion (4 per cent) of lines which were similar in size, form and fission rate to the parent clone.

From these observations and experimental results the conclusion is drawn that the variation is produced by mutations of one member of the four or five heterozygous pairs of genes which are necessary to produce the normal type of this clone.

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